INFUSION CLONING PROTOCOL: BD BIOSCIENCES®

This protocol is adapted from a BD Biosciences protocol by the Gene Expression Lab.
This protocol is for use with BD Biosciences BD In-Fusion PCR Cloning. For additional technical inquiries, contact Technical Service at 877-232-8995 or www.bdbiosciences.com

BEFORE STARTING THE EXPERIMENT

- Always perform procedure using sterile conditions, change the diapers before starting, wipe off pipettes and pens & anything used w/ ETOH.
- Thaw purified PCR Samples and other ingredients on ice.

INFUSION CLONING PROTOCOL

1) Cloning Reaction:

Gene	Tube ID	10X InFusion Rxn Buffer	10x BSA (500ug/ml)	Linearized Vector-100ng/ul	Infus. Enz.	PCR Product (50-200ng)	Water	Amt Ligation to add	# col's
A	1	2ul	2ul	1ul	1ul	n ul	Bring total vol. to 20ul	1ul (if conc. of PCR product is <50ng can use up to 2ul)	

** Make diluted enzyme when using it; do not make ahead of time **

2) Incubate reactions at room temperature for 30 min, and then transfer tubes to ice.
3) Proceed with Transformation. If you cannot transform cells immediately, store at –20 until you are ready.
4) Thaw, on ice, one tube of DH5-T1 Max Efficiency Competent Cells for each ligation/transformation.

5) Pipette designated amt of each ligation reaction directly into the competent cells and mix by tapping gently. **Do not mix by pipetting up and down**. Store the remaining ligation at –20.
6) Incubate vial on ice for 30 min.
7) Incubate for exactly 45 seconds in 42°C water bath. Do not mix or shake.
8) Remove vial from 42°C water bath and place on ice. Add 500ul of pre-warmed SOC medium to each vial. (Sterile technique must be used to avoid contamination.)
    - Wipe SOC w/ ETOH and kimwipes
    - Run SOC through burner flame before opening and after opening
    - Run pipette tip through flame
    - Run sample tube through flame before and after opening
    - Add the 500ul
    - Run sample tube through flame again before and after closing
    - Replace sample tube on ice
    - Repeat for each sample.
9) Shake vial at 37 degrees for exactly 1 hour at 225rpm in a shaking incubator.
10) Take out of incubator and spin in microcentrifuge for 2 min. at 10,000rpm.
11) Remove 400ul of supernatant and discard. Disrupt pellet by tapping gently, make sure pellet has mixed into medium
    - Again use sterile technique
    - Run tip through flame, and run sample tube through flame before and after opening
    - Remove the 400ul and discard in trashcan
    - Run tube and cap through flame before and after closing
    - Gently tap to dissolve pellet into the remaining~100ul
12) Spread rest from each transformation vial on separate labeled plates.
    - Burn glass pipette into L-shape, burn end shut and place upright to cool off
    - To remove remaining 100ul from sample tube, briefly run tip through flame and tube before and after opening
    - Remove 100ul and put onto plate
    - Use glass pipette to spread
13) Invert the plates and incubate at 37 degrees O/N.

TROUBLESHOOTING

No Colony Growth

- It is possible that there was not enough purified PCR product used. The BD Infusion Cloning protocol recommends between 50 and 200ng of purified PCR product. If there is not this much purified product, it may be necessary to repeat the original PCR to get enough amplified

product. An alternate approach would be to reduce the amount of vector so that the ratio of vector:insert is optimal.

- It is possible that the transformation was performed using too much of the cloning reaction. No more than 1-2ul of the cloning reaction mix shoudl be used in the transformation. Or it may be necessary to dilute the cloning reaction mix.

Satellite colony growth

- Do not pick these colonies, as they are antibiotic-sensitive colonies that lack plasmid. It may be necessary to either increase the amount of antibiotic in your selection plates. It is also important to make sure plates / antibiotic solutions are fresh.